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Host plant traits play a crucial role in shaping the composition of epiphytic microbiota in the arid desert, Northwest China

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Abstract: Phyllosphere microorganisms are a crucial component of environmental microorganisms, highly influenced by host characteristics, and play a significant role in plant health and productivity. Nonetheless, the impact of host characteristics on shaping phyllosphere microbial communities of plants with different life forms remains ambiguous. Utilizing high-throughput sequencing technology, this study analyzed the diversity and community composition of phyllosphere epiphytic microorganisms (e.g., bacteria and fungi) of various plant life forms in the hinterland of the Gurbantunggut Desert, Northwest China. Functional annotation of prokaryotic taxa (FAPROTAX) and fungi function guild (FUNGuild) were employed to assess the ecological functions of microorganisms and to investigate the role of stochastic and deterministic processes in shaping phyllosphere microbial communities. Result showed a diverse array of phyllosphere epiphytic microorganisms in the desert plants, with Proteobacteria, Cyanobacteria, and Actinobacteriota dominating bacterial community, while Ascomycota and Basidiomycota were prevalent in fungal community. Comparison across different plant life forms highlighted distinct microbial communities, indicating strong filtering effects by plant characteristics. FAPROTAX prediction identified intracellular parasites (accounting for 27.44% of bacterial community abundance), chemoheterotrophy (10.12%), and phototrophy (17.41%) as the main functions of epiphytic bacteria on leaves of different life form plants. FUNGuild prediction indicated that phyllosphere epiphytic fungi primarily served as Saprotrophs (81.77%), Pathotrophs (17.41%), and Symbiotrophs (0.82%). Co-occurrence network analysis demonstrated a predominance of positive correlations among different microbial taxa. Raup-Crick dissimilarity index analysis revealed that deterministic processes predominantly influenced phyllosphere bacterial and fungal community assembly. Variance partitioning analysis and random forest modeling suggested that plant leaf functional traits significantly impacted both bacterial and fungal community composition, with fungal community composition showing a closer association with leaf nutrients and physiology compared with bacterial community composition. The distinct responses of bacterial and fungal communities to plant traits were attributed to the differing properties of bacteria and fungi, such as bacteria having higher potential dispersal rates and broader ecological niches than fungi. Overall, the results indicate that phyllosphere bacterial and fungal communities undergo similar community assembly processes, with fungi being more influenced by plant characteristics than bacteria. These findings offer novel insights into the ecology of phyllosphere microbial communities of desert plants.

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1 Introduction

Phyllosphere, which refers to the surface of above-ground plant organs (primarily leaves), is a significant habitat for microorganisms but often overlooked by humans (Vacher et al., 2016; Laforest-Lapointe and Whitaker, 2019). According to statistics, the collective leaf area of all plants on the Earth exceeds 1.02×10^9 km^2 (Vorholt, 2012). Phyllosphere is typically enclosed by leaf epidermis, which restricts the release of water and nutrients from leaves and creates a nutrient-poor niche (Schreiber, 2010; Thapa et al., 2018). Desert ecosystem presents an extremely harsh environment with stress factors such as intense ultraviolet light, high temperature, drought, and reactive oxygen species in phyllosphere (Rico et al., 2014; Muller et al., 2016). Consequently, it was previously believed that phyllosphere of desert plant would be unsuitable for microbial survival and reproduction. However, recent studies have demonstrated that diverse epiphytic microbial communities thrive in phyllosphere of desert plants. These communities promote plant health while playing essential roles in energy flow and nutrient cycling within ecosystems (Agoussar and Yergeau, 2021). Plants provide specialized niches for their epiphytic microorganisms to meet their nutritional requirements (Bulgarelli et al., 2013; Gong and Xin, 2021). Nevertheless, different plants offer distinct niches along with varying food sources and secondary metabolites to their phyllosphere microorganisms, resulting in the recruitment of unique microbial communities (Wagner et al., 2016; Xiong et al., 2021). In arid deserts, phyllosphere serves as a refuge for microorganisms (Hakobyan et al., 2023; Liu et al., 2023). The investigation of phyllosphere microorganism diversity in desert plants contributes to a more comprehensive understanding of the factors influencing plant characteristics that shape phyllosphere microbial diversity.

Diversity of plant phyllosphere microorganisms is closely linked to variations in functional traits of plant leaves (Kembel et al., 2014). Yadav et al. (2005) discovered that water content and phosphorus concentration in plant leaves have a notable impact on the abundance of phyllosphere bacteria. Relevant research confirmed that nitrogen and phosphorus contents in tree leaves notably influence the composition of phyllosphere bacterial communities (Kembel et al., 2014), whereas aluminum concentration significantly impacts the composition of phyllosphere fungal communities (Kembel and Mueller, 2014). Moreover, Hunter et al. (2010) found that the composition of phyllosphere bacterial community in lettuce (*Lactuca sativa* Linn.) correlates with leaf morphology and calcium element. A positive correlation has been identified between nitrogen content of oak (*Quercus petraea* (Matt.) LieBlume) leaves and the composition of leaf bacterial communities (Borruso et al., 2018). Furthermore, plant genotypes and interspecies interactions also play a role in shaping the composition of phyllosphere microbial community (Laforest-Lapointe et al., 2016; Li et al., 2021). For instance, in desert environments, microorganisms establish intricate interaction networks involving symbiosis, mutualism, competition, coexistence, and antagonism within the community (Liu et al., 2023). These complex networks can influence and maintain microbial community structure and interaction dynamics, thereby significantly impacting the stability of desert ecosystems (Yin et al., 2023). Recent studies have utilized co-occurrence network analysis to validate the connections among microbial community members, unveil the network characteristics of each operational taxon within community, and pinpoint key taxa and their roles in member interaction and community composition (He et al., 2017; Rottjers and Faust, 2018). These studies indicate that plant genotypes, functional traits, and interspecies interactions collectively influence the composition of phyllosphere microbial community. Nonetheless, it remains uncertain whether plant life forms will impact the composition of phyllosphere microbial community.

The Gurbantunggut Desert, located in Central Asia, is a typical temperate desert characterized by distinct variations in water availability and temperature, as well as a diverse range of plant species (Qian et al., 2010). Serving as a crucial repository of plant germplasm resources in China, this desert has wide distribution of plant life forms including small trees, shrubs, and herbs, all of which play a vital role in ecological functions such as windbreak and sand fixation (Zhang and Chen, 2002). Phyllosphere of different plant life forms in the desert harbors specific microbial groups that contribute significantly to host health and ecosystem stability. Through the use of high-throughput sequencing technology, this study analyzed plant phyllosphere microorganisms in the Gurbantunggut Desert, Northwest China, to explore the diversity characteristics and impacts of these microbial communities on different plant life forms. The findings of this research serve as a foundational framework for further investigation into desert ecosystem biodiversity. Building upon this study, the following hypotheses were proposed: (1) distinct differences exist in the structure, alpha diversity, and network topology of phyllosphere microbial communities among various plant life forms; and (2) functional traits of plant leaves exert a significant influence on the composition of bacterial and fungal communities, albeit with variations in their effects on each.

2 Materials and methods

2.1 Study area

The study area is located in the hinterland of the Gurbantunggut Desert, Northwest China (44°52′18″N, 87°49′39″E). It has a temperate continental arid climate, with an annual average temperature of 6.57℃. In summer, the hottest month (July) has temperature ranging from 25.50℃ to 30.30℃, while in winter, the coldest month (January) has temperature ranging from –22.70℃ to –26.80℃. The area receives an annual average precipitation of 70–180 mm, but evaporation exceeds 2000 mm, approximately 11 to 28 times higher than precipitation. From late November to mid-March, the Gurbantunggut Desert landscape is covered by snow.

In mid-March 2022, we carefully selected sample plots within the study area, ensuring vegetation was evenly distributed. Six quadrats with an area of 30 m×30 m were then established with a spacing of 5 m between each quadrat. Following the methodology delineated by Qian et al. (2010), the desert vegetation within each quadrat was categorized into three life forms: Tree (Tr), Shrub (Sh), and Herb (He). Two dominant plants from each life form were chosen, as described in Table 1. Subsequently, in mid-May 2022, desert plants with identical ground diameter, height, and crown size across different life forms were selected. Leaves were then collected from the east, south, west, north, and center directions of each plant's crown, with leaves from the same plant within a quadrat being combined into a single sample. This process resulted in a total of 6 replicates for each plant, following the approach of Huang and Li (2015). Soil conditions within the 0–20 cm soil layer of each quadrat were found to be uniform. Specifically, soil has a pH of 8.11 (±0.08), a moisture content of 0.42% (±0.05%), soil organic carbon content of 0.45 (±0.04) g/kg, nitrate content of 0.45 (±0.04) g/kg, nitrogen content of 2.44 (±0.16) mg/kg, ammonia nitrogen content of 5.36 (±0.27) mg/kg, and available phosphorus content of 4.16 (±0.35) mg/kg.

Table 1 Characteristics of various life-form plants

Life form and code	Species and code	Base diameter (cm)	Plant height (m)	Crown area (m^2)
Tree (Tr)	*Haloxylon ammodendron* (C.A.Mey.) Bunge (Ha)	1.55±0.23	1.25±0.25	0.51±0.14
	Haloxylon periscum Bunge ex Boiss & Buhse (Hp)	1.51±0.31	1.24±0.32	0.54±0.13
Shrub (Sh)	*Calligonum caput-medusae* Schrenk (Cc)	1.57±0.35	1.25±0.14	0.56±0.12
	Calligonum leucocladum (Schrenk) Bunge (Cl)	1.52±0.24	1.24±0.23	0.55±0.09
Herb (He)	*Astragalus cognatus* Schrenk (Ac)	1.48±0.32	1.19±0.21	0.56±0.11
	Astragalus steinbergianus Sumn (As)	1.47±0.28	1.18±0.32	0.57±0.14

Note: Mean±SD.

2.2 Field sample collection and phyllosphere microbial elution

Collectors wear latex gloves and sterilize their hands and tools with 75% alcohol. When collecting leaves, they trim them carefully from plant canopy, place them in sterile collection bags, and seal the bags promptly. Samples are then stored in a freezer for preservation. Upon returning to laboratory, leaf samples are immediately transferred to a –80℃ ultra-low temperature refrigerator for storage.

In the ultra-clean workbench, a leaf sample weighing 5 g was measured using a precision balance with an accuracy of 1/1000. Sample was then transferred into an aseptic tissue culture bottle with a volume of 270.0 mL. Subsequently, 50.0 mL of sterile phosphate buffer solution (containing 0.24 g KH_2PO_4, 1.44 g NaH_2PO_4, 8.00 g NaCl, and 2.00 g KCl per liter at pH=7.40) was added to the bottle. Solution was agitated using a shaker operating at a speed of 200 r/min for 30 min at room temperature. After removing any visible impurities, we carefully transferred the turbid liquid to a centrifuge tube with a capacity of 50.0 mL. Tube was then centrifuged at a rotational speed of 10,000 r/min for 10 min. Following this step, the supernatant was discarded while microbial sediment settled at the bottom of tube was carefully transferred to another sterile centrifuge tube with a capacity of 1.5 mL. This transfer was done in order to proceed with subsequent microbial deoxyribonucleic acid (DNA) extraction.

2.3 DNA extraction and amplicon sequencing of phyllosphere microorganisms

According to the method of a genomic DNA extraction kit (Tiangen biotech CO., LTD., Beijing, China), we extracted the DNA of phyllosphere microorganisms. Concentration of microbial DNA was determined using an enzyme marker (Synergy HTX, Bio-Tek, Winooski, USA). We designed primers based on conserved region sequences and added a sequencing connector to the end for polymerase chain reaction (PCR) amplification. The products were purified, quantified, and standardized. To ensure the quality of microbial DNA samples for database construction, the target band should have a significantly stronger intensity than any non-specific bands if the size of target band is correct and the fragment is less than 550 bp. We analyzed the library using a high-throughput biofragment analyzer (Qsep400, BiOptic Inc., New Taipei, China) and then performed sequencing on the Illumina NovaSeq 6000 platform. For bacterial 16S (V3+V4) amplification, we used universal primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3'), while for fungal internally transcribed spacer (ITS1) amplification, we used universal primers ITS1F (5'-CTTGGTCATTTAGAGGAAGTAA-3') and ITS2R (5'-GCTGCGTTCTTCATCGATGC-3') (Walters et al., 2016). PCR reaction conditions included an initial denaturation at 95℃ for 5 min, denaturation at 95℃ for 30 s, annealing at 50℃ for 30 s, extension at 72℃ for 40 s for a total of 30 cycles, and a final extension at 72℃ for 7 min.

Data information analysis process is as follows: (1) quality filtering: the raw reads were filtered using Trimmomatic v.0.33 software. Primer sequences were identified and removed using Cutadapt v.1.9.1 software, resulting in clean reads without primer sequences; (2) double-ended sequence splicing: Usearch v.10.0 software was utilized to splice clean reads of each sample through overlap. Then we filtered the spliced data based on the length range of different areas; and (3) chimaera removal: UCHIME v.4.2 software was employed to identify and eliminate chimera sequences, obtaining effective reads.

2.4 Determination of plant functional traits

Leaf area tester (YMJ-C, Zhejiang Topu Instrument Co., Ltd., Hangzhou, China) was utilized to measure blade length (BL), blade width (BW), and leaf area (LA) of plant. Subsequently, the leaves were dried in an oven at 65°C until a constant weight, and dry weight was recorded. Specific leaf area (SLA) was calculated by dividing leaf area by dry weight. Dried leaves were then finely crushed and sieved through a 0.25-mm screen. Soluble sugar (SS) and starch (ST) contents in the leaves were determined using improved phenol-sulfuric acid method in two steps as described by Guo et al. (2004). Total carbon (TC) and nitrogen (TN) contents in the leaves were analyzed using a carbon and nitrogen analyzer (Multi N/C 2100S, Analytik Jena AG, Jena,

Germany) (Wu et al., 2014). We determined total phosphorus (TP) content in the leaves using molybdenum-antimony resistance colorimetry, and total potassium (TK) content using the method of Yue et al. (2017). Total phenols (TPH) and total flavonoids (TF) in the leaves were assessed following the method of Vidhyasekaran et al. (1992).

2.5 Data analysis

Shapiro-Wilk test was used to assess the normality of data before conducting statistical analysis. If the data did not follow a normal distribution, a logarithmic transformation was applied to approximate normality. Microorganisms with a relative abundance greater than 0.1% were screened, and the 'networkD3' package in the R language was used to generate a Sankey map illustrating the composition of microbial community. One-way analysis of variation (ANOVA) was performed to analyze the relative abundance and alpha diversity index of phyllosphere epiphytic microbial communities across different plant life forms and plant species. We assessed the similarity of phyllosphere epiphytic microbial communities using principal coordinate analysis (PCoA). This analysis, combined with permutation-based multivariate ANOVA (PERMANOVA), determined whether there were noticeable differences in microbial community structure between samples. We selected the top 15 genera with the highest abundance using the 'stat' package in the R language, based on the obtained microbial abundance data. Hypothesis testing between microbial communities of different plant life forms or plant species was conducted using an inter-group difference test method and rigorous statistical approaches to evaluate the significance of species abundance differences. Species information that showed significant differences among various plant life forms and plant species was obtained. To investigate the correlation between levels of microbial operational taxonomic units (OTUs), we constructed a network co-occurrence map after combining OTUs with an abundance less than 0.1%. The threshold value for this combination is determined by calculating Spearman correlation coefficient and Jaccard distance (Yuan et al., 2021). To calculate species, we used correlation *P* values below 0.05 after correcting them with false discovery rate. We used the R language packages 'Hmisc' and 'igraph' along with interactive platform Gephi for visualization. Functional annotation of prokaryotic taxa database is a meticulously curated resource for prokaryotic functional annotation, which involves compiling bacterial literature, developing bacterial species classification and functional annotation, and categorizing bacterial functions. The FUNGuild employs bioinformatics techniques to integrate species classification with functional guild classification, providing a comprehensive system for fungal functional classification. Variance partitioning analysis (VPA) was conducted using the 'varpart' function from 'vegan' package. Random forest model was constructed with the 'randomForest' package, and the significance of percentage that increased in mean squared error (MSE) for each predictor was assessed using the 'rfPermute' package along with the 'A3' package.

3 Results

3.1 Quality assessment of sequencing data

Quality assessment of sequencing data revealed that bacterial and fungal reads for the 36 samples were 280,569 and 2,875,496 pairs, respectively. After conducting quality control and splicing procedures, we obtained a total of 2,873,414 clean reads for bacteria and 2,864,822 clean reads for fungi. On average, each sample yielded approximately 79,817 clean reads for bacteria and 79,578 clean reads for fungi. Using Usearch software, we clustered sequences at a similarity level of 97% and identified a total of 464 OTUs for bacteria and 544 OTUs for fungi. The dilution curve showed that the number of OTUs remained stable as more bacteria and fungi were sequenced, indicating that the sequencing depth met the requirements for diversity analysis.

3.2 Abundance of phyllosphere microbial community

Results showed that Proteobacteria, Cyanobacteria, Actinobacteriota, and Firmicutes were the

dominant phyla among phyllosphere epiphytic bacteria in different plant life forms. These four phyla accounted for a total of 63.05% of the bacterial community structure (Fig. 1). The dominant phyllosphere epiphytic bacteria belonged to 4 phyla, 5 classes, 10 orders, 15 families, and 9 genera. Among these genera, *Acinetobacter* (F=7.8988, P=0.0051), *Arthrobacter* (F=5.9481,

Fig. 1 Community structure of epiphytic bacterial communities (a) and fungal communities (b) of desert plants

P=0.0135), and *Geodermatophilus* (*F*=4.7981, *P*=0.0241) showed significant variations across different plant life forms (Fig. 2). Similarly, significant differences were also observed among different plant species (Fig. Sl). Abundance of bacterial genera varied among different plant life forms, with He>Sh>Tr. However, variation in their abundance among plant species did not follow a regular pattern.

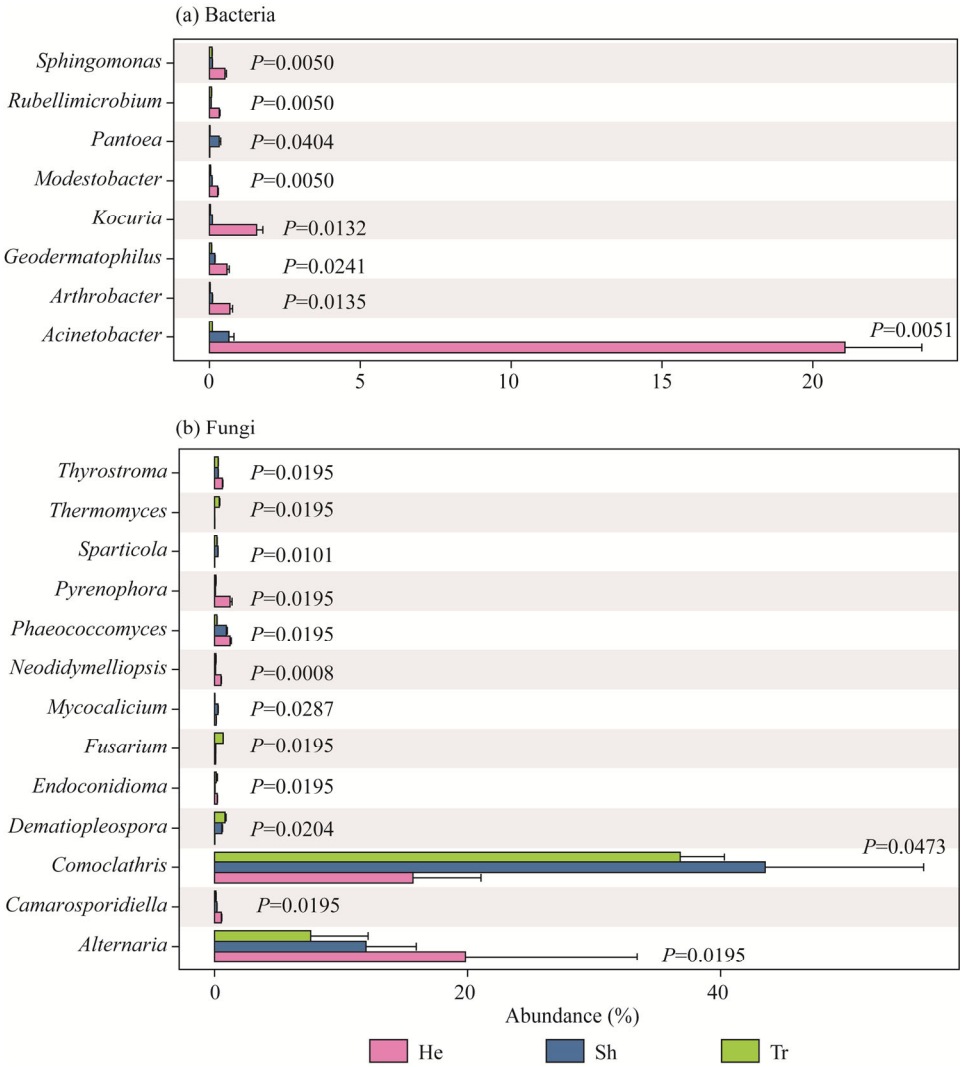

Fig. 2 Abundance of epiphytic bacterial communities (a) and fungal communities (b) of different plant life forms at genus level. Bars are standard errors. He, herb; Sh, shrub; Tr, tree. The abbreviations are the same as in the following figures.

Results found that dominant phyla in phyllosphere epiphytic fungi were Ascomycota and Basidiomycota, accounting for a total of 74.30% of the fungal community structure (Fig. 1). These fungi belonged to 2 phyla, 6 classes, 14 orders, 21 families, and 40 genera. Among them, the abundance of *Comoclathris* (*F*=6.2701, *P*=0.0195), *Alternaria* (*F*=6.6301, *P*=0.0950), and *Camarosporidiella* (*F*=4.0118, *P*=0.0473) showed significant differences among different plant life forms. Similarly, significant differences were also observed among different plant species. Abundance of *Alternaria*, *Camarosporidiella*, and *Phaeococcomyces* followed a consistent pattern across different plant life forms, with He>Sh>Tr (Fig. 2). Likewise, abundance of *Endoconidioma*, *Neodidymelliopsis*, *Pyrenophora*, and *Thyrostroma* showed certain trends among different plant life forms, with He>Tr>Sh. However, abundance of these fungal genera did not

display regular variations across plant species (Fig. Sl).

3.3 Alpha diversity of epiphytic microbial community

In addition to the richness index, significant differences were observed in Shannon and Pielou evenness indices of phyllosphere bacterial communities of different plant life forms. Shannon and Pielou evenness indices of phyllosphere bacterial communities of He were significantly higher than those of Tr. They did not differ significantly between He and Sh, and between Tr and Sh (Fig. 3). Richness, Shannon, and Pielou evenness indices showed significant differences among phyllosphere bacterial communities of different plant species. *Haloxylon periscum* Bunge ex Boiss & Buhse (Hp) had the highest richness index, while *Haloxylon ammodendron* (C.A.Mey.) Bunge (Ha) had the lowest value. In terms of both Shannon and Pielou evenness indices, *Astragalus cognatus* Schrenk (Ac) had the highest values, while Ha had the lowest values (Fig. S2).

Results showed that significant variations in the richness and Pielou evenness indices of phyllosphere fungal communities of different plant life forms, except for Shannon index. Notably, Pielou evenness index was higher in He and Sh compared with Tr, while the richness index was lower in He and Sh compared with Tr. There was no significant difference between the richness and Pielou evenness indices of phyllosphere fungal communities of He compared with Sh (Fig. 3). Furthermore, both the richness and Pielou evenness indices showed significant variations among different plant species. The highest richness index was found in Hp, while Ha had the lowest value. In terms of Pielou evenness index, Ac had the highest value, while Ha had the lowest value (Fig. S2).

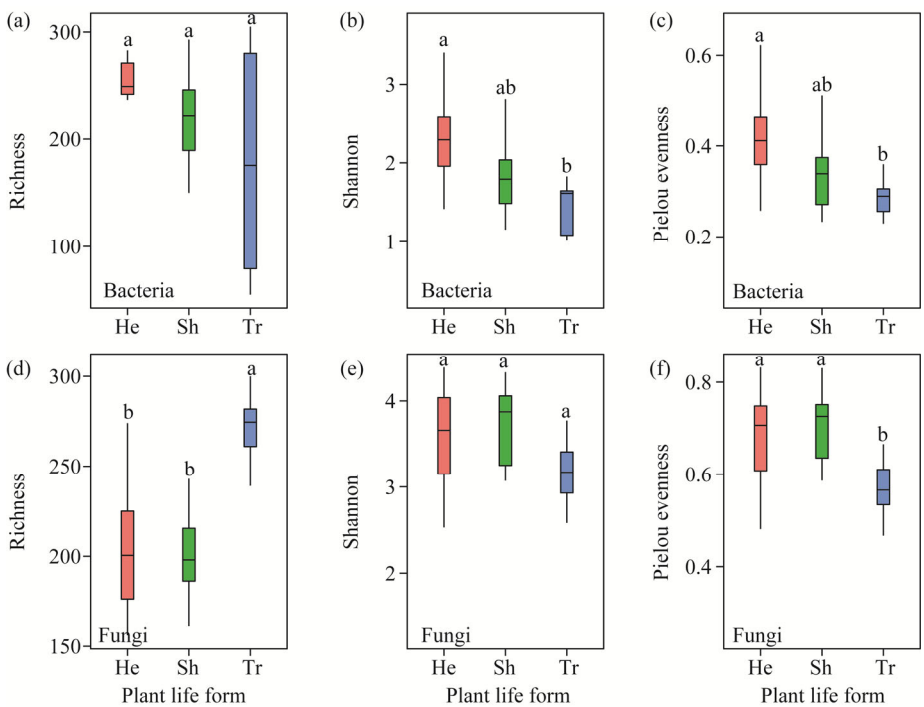

Fig. 3 Alpha diversity of epiphytic bacterial communities (a–c) and fungal communities (d–f) of different plant life forms. Different lowercase letters indicate significant differences among different plant life forms at $P<0.050$ level. Boxes indicate the IQR (interquartile range, 75th to 25th of the data). The median value is shown as a line within the box. Lines extend to the most extreme value within 1.5×IQR.

3.4 Difference in community composition of phyllosphere epiphytic microorganisms

Six desert plants (e.g., Ac, *Astragalus steinbergianus* Sumn (As), *Calligonum caput-medusae* Schrenk (Cc), *Calligonum leucocladum* (Schrenk) Bunge (Cl), Ha, and Hp; Table 1) contained a total of 125 common bacterial OTUs. Each plant hosted different number of unique bacterial

OTUs, with Cc having the highest number (32), followed by As (5), Ha (4), Cl (3), Ac (2), and Hp (2) (Fig. 4). Furthermore, these plants contained a total of 207 common fungal OTUs, with Hp having the largest number (9), followed by Ha (8), As (8), Cc (5), Cl (5), and Ac (2) (Fig. 4).

Phyllosphere of desert plants contains a unique and diverse structure of epiphytic bacteria. PCoA indicated that principal coordinate 1 (PC1) and PC2 explained 60.41% and 18.15% of the variation in the epiphytic bacterial community, contributing to a cumulative explanation of 78.56%. PERMANOVA analysis indicated that plant characteristics accounted for 71.05% of the variation in bacterial communities. Similarly, plant life forms were found to explain 66.12% of the variation in bacterial communities (Fig. 4).

Results showed that the unique and distinct structure of epiphytic fungi. PCoA indicated that PC1 and PC2 explained 47.48% and 15.79% of the variation in the epiphytic fungal community, with a cumulative explanation of 63.27%. PERMANOVA analysis revealed that plant characteristics accounted for 55.17% of the variation in fungal communities, while plant life forms explained 27.35% of the variation (Fig. 4).

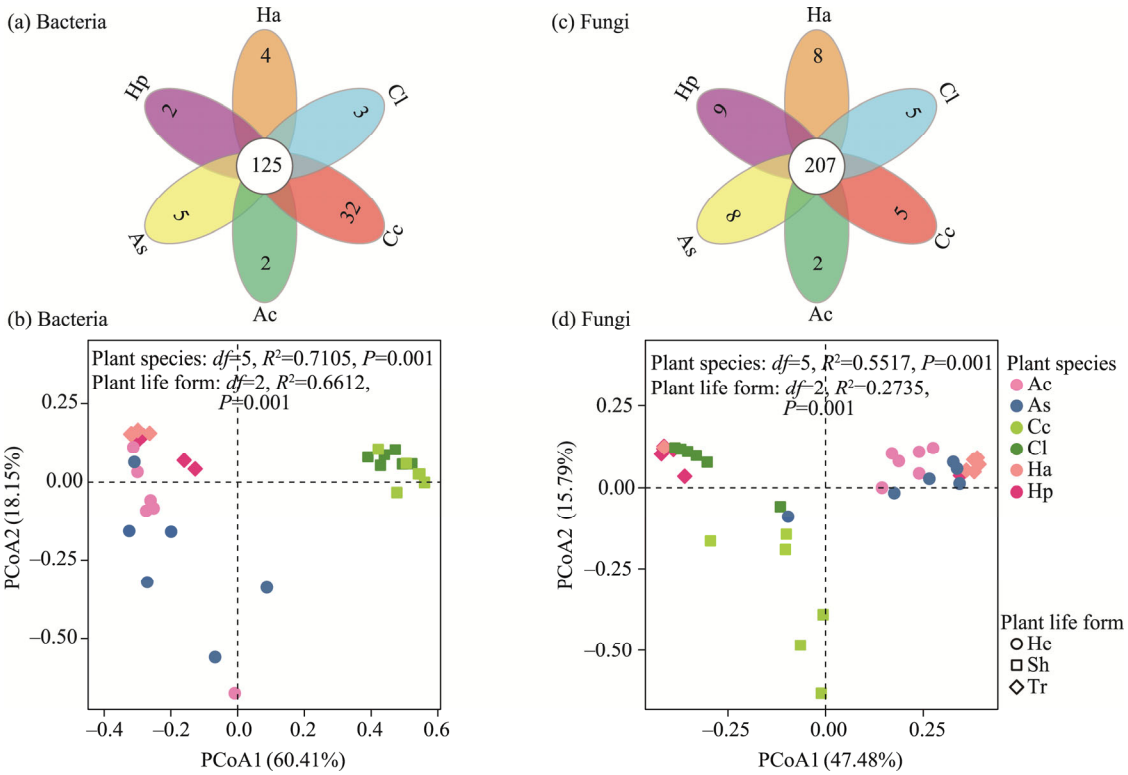

Fig. 4 Venn diagram and principal coordinates analysis (PCoA) of epiphytic bacterial communities (a, b) and fungal communities (c, d). Ha, *Haloxylon ammodendron* (C. A. Mey.) Bunge; Hp, *Haloxylon periscum* Bunge ex Boiss & Buhse; Cc, *Calligonum caput-medusae* Schrenk; Cl, *Calligonum leucocladum* (Schrenk) Bunge; Ac, *Astragalus cognatus* Schrenk; As, *Astragalus steinbergianus* Sumn.

Bacterial communities in He, Sh, and Tr had a higher species richness than expected by random selection, indicating a deterministic assembly process (Fig. 5). Similarly, bacterial communities in Ac, As, Cc, Cl, Ha, and Hp also displayed a greater species richness than expected by random selection, suggesting a deterministic assembly process (Fig. S3). For fungi, the deterministic assembly process in He, Sh, and Tr showed an increased species richness compared with random selection (Fig. 5). Additionally, the deterministic assembly process of fungal communities in Ac, As, Cc, Cl, Ha, and Hp also had more species than expected by random selection (Fig. S3).

3.5 Phyllospheric microbial co-occurrence patterns and ecological functions

To investigate the impact of plant life forms on potential interactions among epiphytic bacterial communities, we analyzed the topologies of epiphytic bacterial networks of He, Sh, and Tr. The

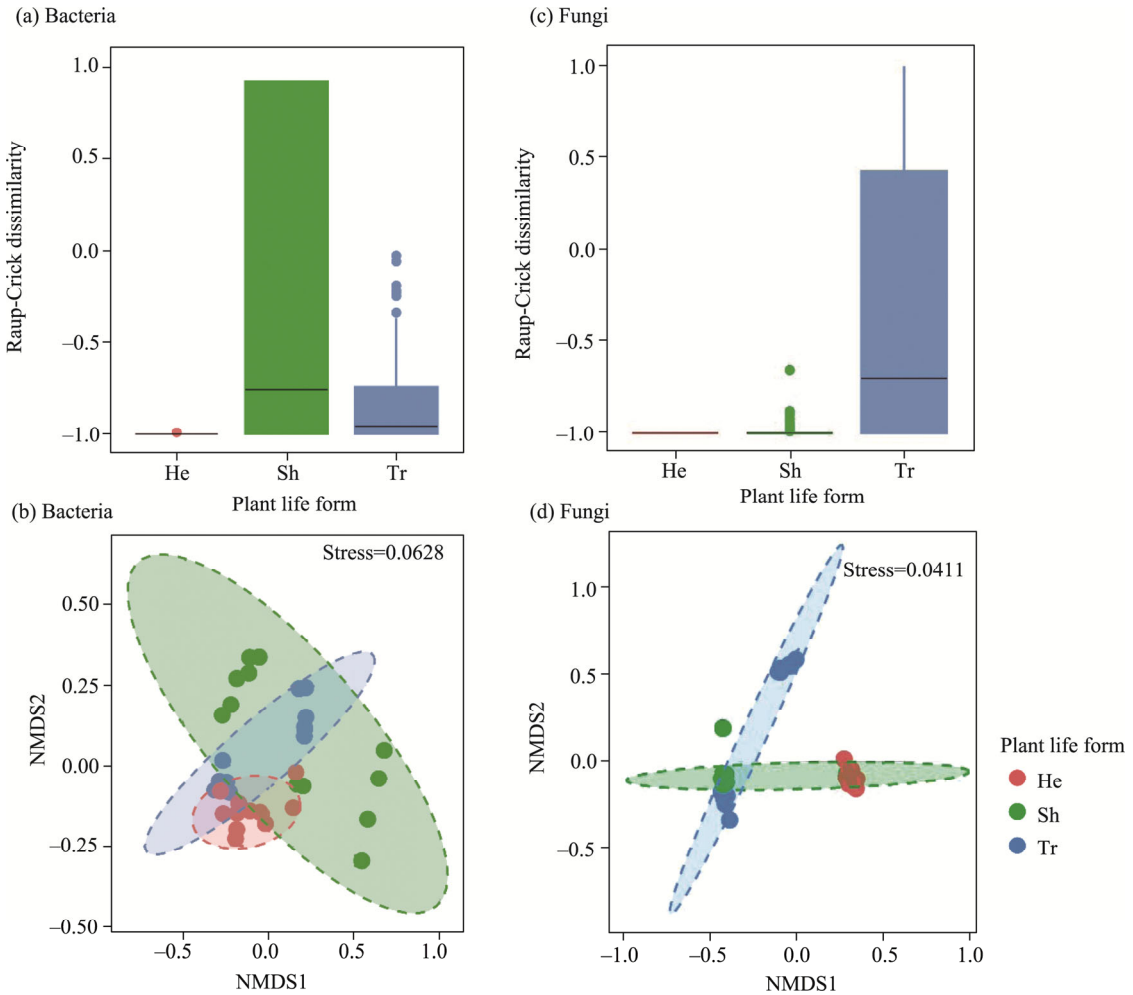

Fig. 5 Raup-Crick dissimilarity index and non-metric multi-dimensional dissimilarity (NMDS) index of phyllosphere epiphytic bacterial communities (a, b) and fungal communities (c, d) of different plant life forms. The median value is shown as a line within the box and outlier is shown as circle in Figure 5a and c.

correlation network of epiphytic bacteria from different life forms exhibited a highly interconnected pattern. Results showed that network complexity of plyllosphere epiphytic bacterial communities across different plant life forms was the following order of Tr>SH>He, and varied among different plant species with the following order of Cl>Cc>Ha>Ac>As>Hp (Figs. S4 and S5; Tables S1 and S2). Meanwhile, leaf epiphytic fungal networks of He, Sh, and Tr were constructed to investigate the impact of plant life forms on potential interactions among different fungal communities. Network complexity of phyllosphere epiphytic fungal communities across different plant life forms was the following order of Tr>He>Sh, and varied among different plant species with the following order of Ac>Cc>Hp>As>Cl>Ha (Figs. S4 and S5; Tables S1 and S2).

Bacterial taxa were functionally annotated using the FAPROTAX database, which resulted in 217 OTUs being assigned to 30 ecological functions within bacterial communities. These functions included phototrophy (9.97%), photoautotrophy (9.87%), oxygenic photoautotrophy (9.87%), cyanobacteria (9.87%), intracellular parasites (27.44%), chemoheterotrophy (10.12%), aerobic chemoheterotrophy (5.35%), animal parasites or symbionts (3.86%), human pathogens (3.84%), and aromatic compound degradation (3.72%). Bacteria with intracellular parasites were more prevalent in He and Tr, while bacteria with phototrophy dominated in Sh. Bacteria with photoautotrophy, oxygenic photoautotrophy, and cyanobacteria accounted for a relatively small proportion in He, while those associated with aromatic compound degradation had a relatively

low abundance in Tr and Sh. Epiphytic bacterial community of He had high functional diversity among different plant life forms compared with those of Tr and Sh, which showed low functional diversity. Interestingly, bacterial function diversity showed similarities among different plant life forms as well as among different plant species with the same life form (Figs. 6 and S6).

A total of 256 OTUs were identified, representing 148 ecological functions within phyllosphere epiphytic fungal communities. We primarily classified these functions based on nutritional mode, with saprotrophs accounting for 81.77%, followed by pathotrophs (17.41%), and symbiotrophs (0.82%). Saprotrophic fungi were dominant in He, SH, and Tr, while symbiotrophic fungi constituted a smaller proportion in these samples. Fungal function diversity showed similarities among different plant life forms as well as among different plant species with the same life form (Figs. 6 and S6).

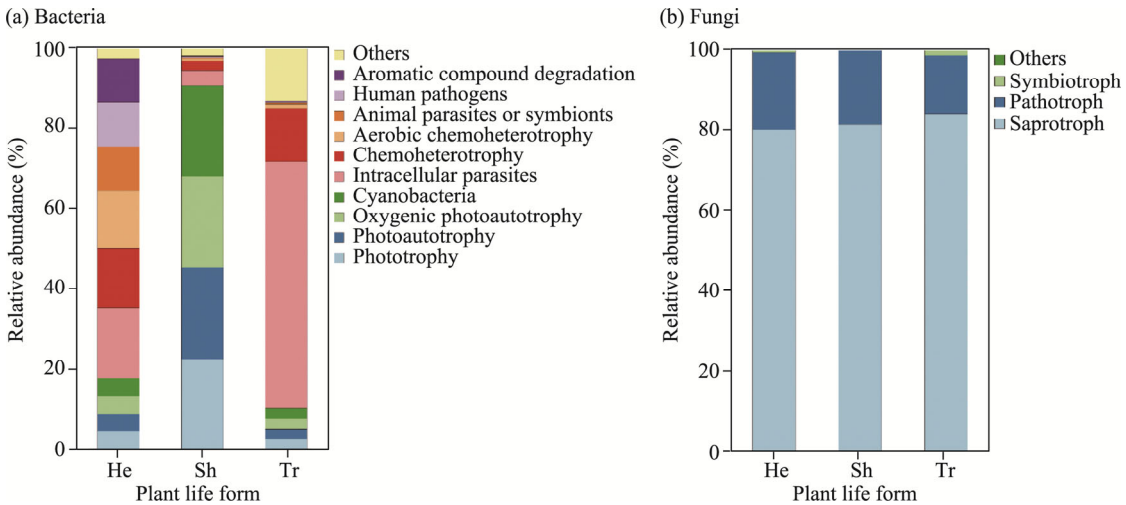

Fig. 6 Function prediction of epiphytic bacterial communities (a) and fungal communities (b) of different plant life forms

3.6 Response of microbial community composition to leaf functional traits

The results indicated significant differences in leaf functional traits among different desert plants (P<0.050; Fig. S7). Spearman correlation analysis revealed that phyllosphere bacterial community was less influenced by plant functional traits compared with fungal community (Table 2). In terms of plant leaf morphological characteristics, LA, BL, BW, and SLA were strongly associated with the abundance of bacterial and fungal communities (P<0.001). BL showed a significant correlation with alpha diversity of fungi (P<0.050), while no significant correlation was found with bacterial alpha diversity. LA, BL, BW, and SLA were significantly linked to the community composition of fungi (P<0.050), but not with the community composition of bacteria. Concerning the nutrient characteristics of plant leaves, TC, TN, TP, and TK were notably correlated with the abundance of bacterial and fungal communities (P<0.001). TC and TK exhibited significant correlations with alpha diversity of fungi (P<0.050), but not with alpha diversity of bacteria. TC, TP, and TK were all significantly associated with fungal community composition (P<0.001), but not with the community composition of bacteria. Physiological characteristics of plant leaves, such as ST, TPH, and TF, showed significant correlation with bacterial community abundance (P<0.001), while SS, ST, and TPH were significantly associated with fungal community abundance (P<0.001). Moreover, SS and ST were significantly linked to alpha diversity of both bacteria and fungi (P<0.050), with SS and ST also showing significant correlations with bacterial community composition (P<0.050) and ST with fungal community composition (P<0.001).

VPA and a random forest model further demonstrated that plant functional traits had a greater impact on fungal community composition than bacterial community composition. Variations in bacterial community composition were largely attributed to the selected plant functional traits, accounting for 69.67% in VPA and 76.00% in the random forest model. On the other hand, the

selected plant functional traits played a more significant role in explaining the variation in fungal community composition, with VPA and random forest models explaining 79.04% and 87.00% of the variation, respectively (Figs. 7 and 8).

Table 2 Spearman's correlation coefficient between bacterial and fungal community properties and leaf functional traits

Bacterial/fungal community property	LA (cm^2)	BL (cm)	BW (cm)	SLA (cm^2/g)	TC (mg/g)	TN (mg/g)
Bacteria						
Abundance	0.312 *P*=0.001	0.526 *P*=0.001	0.486 *P*=0.001	0.640 *P*=0.001	0.313 *P*=0.001	0.177 *P*=0.004
Alpha diversity	–0.094 *P*=0.812	–0.039 *P*=0.667	–0.091 *P*=0.836	–0.096 *P*=0.975	0.068 *P*=0.203	0.029 *P*=0.325
Community composition	0.161 *P*=0.353	0.203 *P*=0.242	0.122 *P*=0.484	0.011 *P*=0.964	–0.244 *P*=0.171	0.074 *P*=0.683
Fungi						
Abundance	0.291 *P*=0.001	0.326 *P*=0.001	0.370 *P*=0.001	0.406 *P*=0.001	0.195 *P*=0.002	0.265 *P*=0.001
Alpha diversity	–0.124 *P*=0.982	0.150 *P*=0.016	–0.070 *P*=0.842	0.006 *P*=0.398	0.435 *P*=0.001	–0.056 *P*=0.775
Community composition	0.373 *P*=0.032	–0.472 *P*=0.001	0.522 *P*=0.001	0.434 *P*=0.010	0.531 *P*=0.001	0.174 *P*=0.312
Bacterial/fungal community property	TP (mg/g)	TK (mg/g)	SS (mg/g)	ST (mg/g)	TPH (mg/g)	TF (mg/g)
Bacteria						
Abundance	0.298 *P*=0.001	0.227 *P*=0.001	0.039 *P*=0.224	0.149 *P*=0.003	0.496 *P*=0.001	0.419 *P*=0.001
Alpha diversity	–0.101 *P*=0.960	0.009 *P*=0.374	0.571 *P*=0.001	0.383 *P*=0.001	–0.036 *P*=0.687	–0.078 *P*=0.925
Community composition	–0.232 *P*=0.182	0.272 *P*=0.114	–0.601 *P*=0.001	0.382 *P*=0.021	–0.222 *P*=0.201	0.011 *P*=0.933
Fungi						
Abundance	0.406 *P*=0.001	0.227 *P*=0.003	0.400 *P*=0.001	0.514 *P*=0.001	0.142 *P*=0.016	0.036 *P*=0.221
Alpha diversity	–0.006 *P*=0.496	0.138 *P*=0.027	0.198 *P*=0.005	0.11 *P*=0.030	0.072 *P*=0.092	0.025 *P*=0.291
Community composition	0.763 *P*=0.001	0.373 *P*=0.031	0.072 *P*=0.691	0.494 *P*=0.001	–0.242 *P*=0.164	–0.183 *P*=0.302

Note: LA, leaf area; BL, blade length; BW, blade width; SLA, specific leaf area; TC, total carbon; TN, total nitrogen; TP, total phosphorus; TK, total potassium; SS, soluble sugar; ST, starch; TPH, total phenol; TF, total flavone. The abbreviations are the same as in the following figures.

4 Discussion

4.1 Taxonomic characteristics

Different ecosystems display notable variations in habitats, leading to distinct structures of microbial communities (Zhao et al., 2019; Liu et al., 2023). Environmental forces play a crucial role in the colonization of microorganisms within plant phyllosphere (Ottesen et al., 2016; Yin et al., 2022). As a result, environmental factors primarily shape microbial communities across diverse ecosystems by influencing nutrient availability and habitat characteristics (Fonseca-García et al., 2016; Wagner et al., 2016). Despite enduring harsh conditions such as high temperatures, aridity, intense ultraviolet radiation, and limited nutrient resources, the microbiome thriving within the phyllosphere exhibits remarkable uniqueness and diversity (Koskella, 2020).

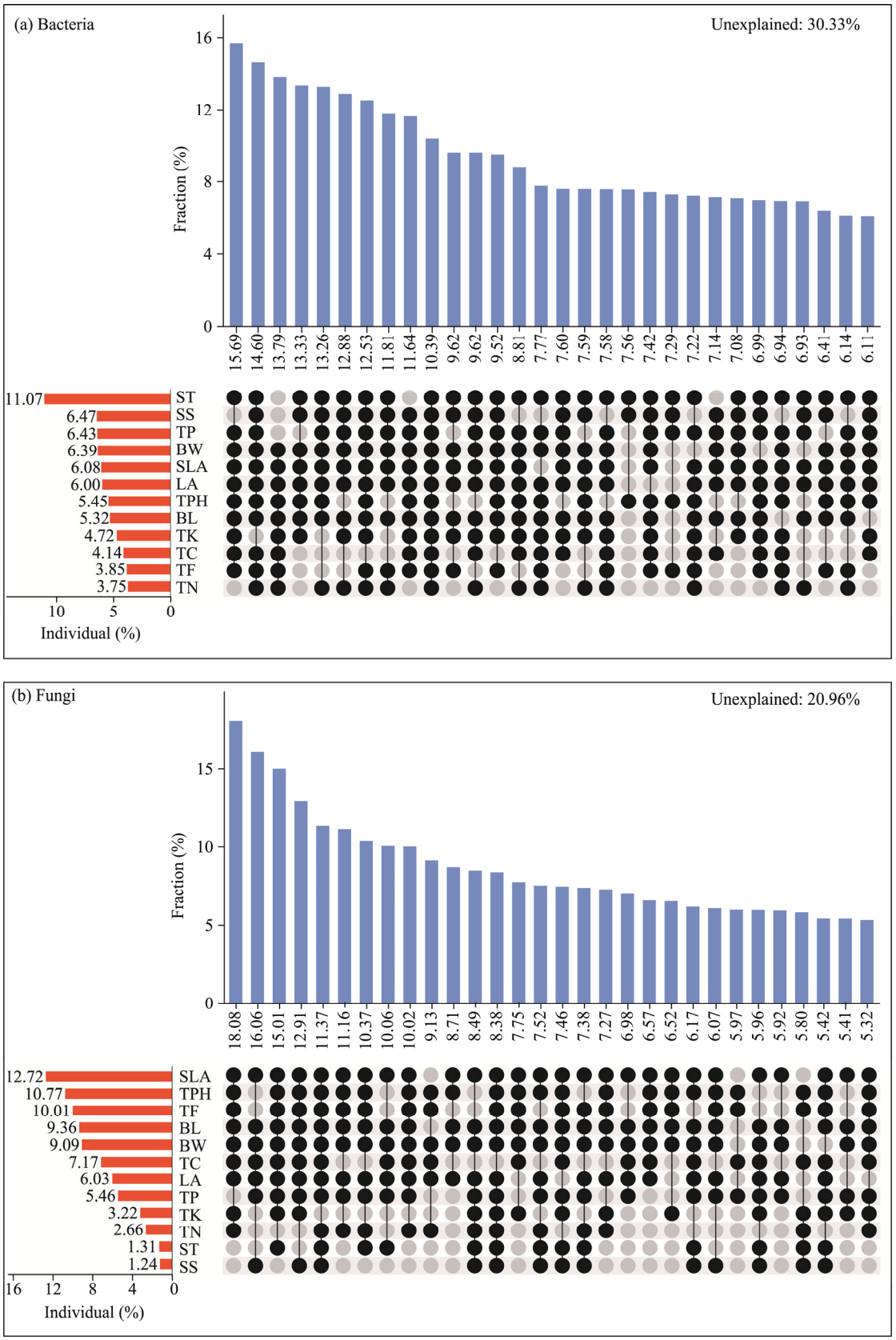

Fig. 7 Variance partitioning analysis and hierarchical segmentation results of canonical analysis of epiphytic bacterial communities (a) and fungal communities (b)

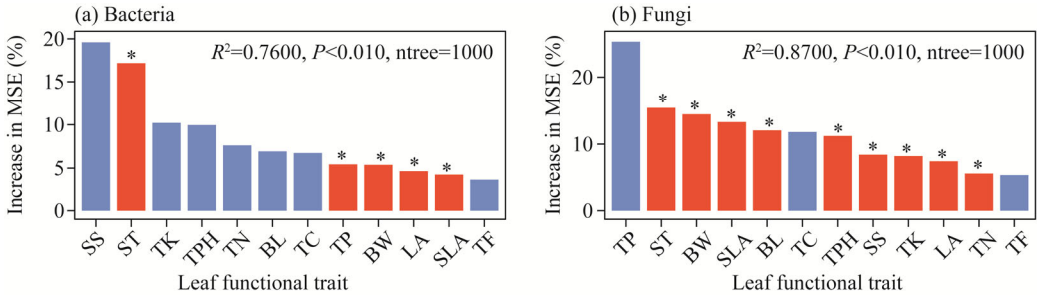

Fig. 8 Importance of random forest modelling environmental factors in predicting epiphytic bacterial communities (a) and fungal communities (b). MSE, mean squared error, *, P<0.050 level.

In order to adapt to arid deserts, plants have undergone specific anatomical modifications in their leaves, including leaf evolution into assimilative branches, thickening of epidermal cells, development of stratum corneum layers, and formation of multiple epidermis layers. These adaptations significantly reduce nutrient sources available for microorganisms (Reich, 2014; Li et al., 2021). Furthermore, the combination of high temperatures, dryness, and intense radiation renders the phyllosphere an extremely challenging habitat for microbes.

The current findings indicate that in arid environments, the phyllosphere of desert plants is dominated by a limited group of bacteria and fungi that are specifically adapted to extreme conditions. Desert epiphytic microorganisms, for example, have been found to harbor more Gram-positive bacteria (Actinomycetes and Firmicutes), and fungi that protect the host from pathogens (Ascomycota), and degrade wood fiber matrix (Basidiomycota). Similar patterns have been observed in other arid areas, such as the desert near the Dead Sea, the Atacama Desert in Chile, the extreme arid area of southern Israel, and the extreme arid desert area of Gansu, China (Finkel et al., 2011; Al Ashhab et al., 2021; Hakobyan et al., 2023; Liu et al., 2023). In line with previous research on farmland ecosystems, which have high abundance of Proteobacteria, our study also found an increase in the community of Proteobacteria. This could be attributed to the fact that thick cell wall in Gram-positive bacteria provides some level of tolerance to drought and strong radiation conditions (Silhavy et al., 2010; Steele et al., 2011). We also found a significant number of unclassified bacteria and fungi within Proteobacteria and Ascomycota, which could be attributed to the limitations of amplicon sequencing and insufficient sequence length (Sorber et al., 2008). Furthermore, our results revealed previously unknown microbial diversity in extremely harsh desert environments. Actinomycete, which are known to produce a large proportion of antibiotics, has been identified in desert plant leaves. Considering that approximately 70% of the reported antibiotics to date are produced by Actinobacteria (Jose and Jebakumar, 2013), desert epiphytic microorganisms may offer a promising avenue for the discovery of new antibiotics (Wilson and Brimble, 2021).

4.2 Characteristics of microbial communities of different plant life forms

In addition to the environment, plant life forms and plant species have the ability to filter epiphytic microbial communities, serving as a secondary filtration system. Affinity associations are formed between plant life forms, plant species, and microbiota (Lajoie and Kembel, 2021). In microbial ecology, the analysis of alpha diversity in amplicon sequencing data is a commonly preferred method for evaluating differences between habitats and is one of the most important features of microbiology (Willis, 2019). Diversity indices are used to quantitatively describe communities, considering the number of species and individuals of different species in a sample or community (Thukral, 2017). In this study, we categorized groups with similar plant morphological and structural characteristics into trees, shrubs, and herbs based on plant life forms. It was observed that Shannon and Pielou evenness indices of phyllosphere epiphytic microbes significantly differed among different plant life forms. Furthermore, at the plant species level, the richness, Shannon, and Pielou evenness indices of phyllosphere epiphytic microbes showed significant differences among different plant species. Variation in the traits of plant species may

selectively influence microbial community diversity.

Our analysis revealed high beta diversity in the interstitial microbial communities of different plant life forms. PCoA sequencing and PERMANOVA results confirmed differences between epiphytic microbial communities of plant life forms, which further verified our hypothesis. Similarly, there was high beta diversity in the inter-phyllosphere microbial communities of various plant species, and PCoA sequencing and PERMANOVA results also confirmed differences between phyllosphere epiphytic microbial communities of plant species. Previous studies have shown that when microbial propagules (e.g., bacteria and fungi) randomly enter the phyllosphere, they fail to colonize the leaves if they are incompatible with plant species, which can be considered as a secondary filtration effect (Lajoie and Kembel, 2021). The degenerating leaves of Tr and Sh are replaced by assimilating branches to perform assimilation functions, which represents the evolution pinnacle of Tr and Sh (Lyshede, 1979). The epidermis of assimilated branches of Tr and Sh appeared smooth and hairless, characterized by thin epidermal cells and cuticle (Gong et al., 2011). In contrast, the leaves of He had hairy surfaces that potentially offered additional space and shade for microorganisms. As a result, the epiphytic microorganism community in He exhibited a higher diversity.

4.3 Symbiotic model and ecological function of phyllosphere epiphytic microbe communities

Symbiosis theory offers a fresh perspective on understanding the interactions between microbial communities. Microbial interactions, which can take the form of mutualism or antagonism, play a significant role in regulating community structure, as well as ecosystem stability and complexity (HilleRisLambers et al., 2012; Williams et al., 2014). Co-occurrence network analysis serves as a valuable tool for investigating the interactions between organisms in microbial communities (Proulx et al., 2005). Positive correlations can be interpreted as mutual symbiosis (Steele et al., 2011) or spatial coincidence, or they may indicate shared nutritional needs among OTUs rather than actual ecological interactions (Barberán et al., 2012). Conversely, negative correlations are generally considered as competition, confrontation, or exploitation (Faust and Raes, 2012). Therefore, it is essential to gather empirical evidence to validate the types of potential interactions. Furthermore, network analysis can uncover species with a high number of network nodes and complex network structures (Xue et al., 2020; Liu et al., 2023). This study revealed that communities of epiphytic microorganisms, such as bacteria and fungi, exhibited predominantly positive correlations. This result suggests that in resource-poor environments like deserts, microbial species not only compete for nutrients and habitat but also engage in mutual collaboration as a survival strategy. Current research findings indicate symbiosis in desert epiphytes, where two species exchange metabolites, benefiting both species. However, Woyke et al. (2006) and Coyte et al. (2015) argue that this may come at the expense of reducing ecological stability. Meanwhile, we found that there were significant differences in the network topological characteristics of phyllosphere microorganisms of different life form plants, which further verified our hypothesis. Food web persistence analysis suggests that the extinction of a species in the network leads to a cascade of local extinctions, indicating that highly modular networks are more favorable for species survival, which helps in preserving community biodiversity and promoting stability (Stouffer and Bascompte, 2011). Additionally, we categorized the microorganisms based on their degree of nodes in the network. The results reveal that taxa with high centrality contribute to maintaining network stability, even though they may not be the most abundant. Previous studies have also shown that high-abundance OTUs are not necessarily more important than low-abundance OTUs, despite their roles in network stability and dynamics (Mandakovic et al., 2018).

Throughout the long-term evolution of plants and their related microorganisms, phyllosphere epiphytic microorganisms enhance plant productivity by influencing host functions and life history, maintaining plant adaptability to the environment, and playing a crucial role in natural material cycle (Xu et al., 2022). Within phyllosphere epiphytic microbial communities, some produce metabolic substances and disrupt hormone signals through competition for limited space and nutrient resources, leading to mutual inhibition (Vorholt, 2012). This study discovered 217

species of epiphytic bacteria in desert plants. These bacteria exhibit various ecological functions such as chemical heterotrophy, photoautotrophy, photoheterotrophy, denitrification, nitrogen fixation, cellulolysis, and fermentation.

There are some pathogenic bacteria, including human pathogens, human gut pathogens, and animal pathogens or symbionts. This result may be attributed to agricultural and grazing activities near the desert. Additionally, we identified a total of 256 species of pathotrophic fungi, symbiotic trophic fungi, and saprotrophic fungi on the desert plants. Pathotrophic fungi comprise animal pathogens and plant pathogens, while fungal symbiotic types include arbuscular mycorrhizal, endophyte, epiphyte, fungal parasite, and lichen parasite. Saprotroph fungi consist of animal parasite, dung saprotroph, soil saprotroph, wood saprotroph, and undefined saprotroph. Previous studies in the forest land and grassland ecosystems have confirmed the functional diversity of phyllosphere microbial communities (Toju et al., 2019; Bechtold et al., 2021; Li et al., 2022; Yan et al., 2022). This study also found significant differences in the functional diversity of epiphytic bacterial communities among different plant life forms, while the functional diversity of fungal communities did not show significant differences. Furthermore, plants with the same life form exhibited similar microbial community functional diversity.

4.4 Factors affecting the structure of phyllosphere microbial community

Phyllosphere harbors a highly diverse microbial community with specific functional characteristics linked to the host (Rosado et al., 2018). Microtopography of plant leaves, influenced by factors like shape, edge wrinkles, and surface protrusions, plays a crucial role in determining the distribution of leaf veins, stomata, and epidermal cell wall connections, thereby shaping the microbial community structure (Hunter et al., 2010). Topography and hydrophobicity, affected by surface wax deposition, can impact local water distribution on leaf surfaces, potentially influencing nutrient availability and microbial composition (Neinhuis and Barthlot, 1997; Lindow and Leveau, 2002). While some studies suggest that leaf morphology like trichomes and leaf area may not significantly impact the phyllosphere microbial community (Kinkel et al., 1987; Reisberg et al., 2012), this study revealed a significant positive correlation between desert plant leaf morphology (e.g., LA, BL, BW, and SLA) and the abundance and composition of both bacterial and fungal communities. Collectively, these findings underscore the close relationship between leaf morphology and phyllosphere microbial community.

Phyllosphere microbial ecosystem is significantly influenced by plant leaf nitrogen levels, impacting both the total number of communities and species composition. Research indicates that lower leaf nitrogen concentrations are associated with a higher abundance of bacteria in the phyllosphere (Laforest-Lapointe et al., 2016). Conversely, high leaf nitrogen content increases the likelihood of disease occurrence, potentially due to nitrogen-induced susceptibility to pathogens (Huang et al., 2017). Studies have demonstrated a significant negative correlation between nitrogen content in tobacco leaves and certain pathogenic fungi, particularly Fusarium and Botryotrichum (Gao et al., 2023). Furthermore, research has highlighted that endophytic bacterial community structure in the phyllosphere of rubber trees (*Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg.) is influenced by factors such as total nitrogen, total phosphorus, and leaf water content (Wei et al., 2022). Additionally, the response to potassium deficiency also impacts representative taxa in the phyllosphere microbial community. Specifically, leaf potassium content shows a significant positive correlation with various fungal microbial communities, including genera like *Apiotrichum*, *Xeromyces*, and *Cutaneotrichospron* (Gao et al., 2023). Our study revealed a significant positive correlation between TC, TN, TP, and TK in leaves with both the abundance of bacterial and fungal communities, which demonstrated a strong connection between phyllosphere microbial community and leaf nutrients.

Previous research has demonstrated that sugar content in the extracellular fluid of various sugarcane varieties can impact the concentration of nutrients present on the plant's surface, thus influencing the abundance of its phyllosphere epiphytic flora (Asis et al., 2003). Studies have indicated that soluble carbohydrate levels play a crucial role in shaping plant phyllosphere microbial community (Hunter et al., 2010). Carbon source availability is generally considered a

primary limiting factor for phyllosphere microbial growth, as compared with mineral nutrition (Lindow and Brandl, 2003). Our study found significant positive correlations of SS and ST in desert plant leaves with the abundance, alpha diversity, and community composition of bacteria and fungi. Moreover, the presence and expression levels of various phenolic compounds have been linked to plant defense mechanisms against pathogenic bacteria and fungi (Treutter, 2006; Korkina, 2007). While most plants host both endophytic and epiphytic bacterial populations without eliciting defense responses, it appears that induced phenolic pathways do not have a broad impact on the overall phyllosphere microbial community (Hunter et al., 2010). Nonetheless, our study identified significant positive correlations of TPH and TF in the leaves with the abundance of bacterial communities, although no significant relationship was observed with alpha diversity and composition of bacterial communities. TPH in the leaves exhibited a significant positive correlation with the abundance of fungal communities, while showing no significant relationship with alpha diversity and composition of fungal communities. These findings suggest a strong connection between phyllosphere microbial community and leaf physiology.

In this study, it was discovered that various plant leaf functional traits (e.g., LA, BL, BW, SLA, TC, TN, TP, TK, SS, ST, TPH, and TF) played a crucial role in shaping the community composition of bacteria and fungi. However, the effects on bacteria and fungi differ. For instance, ST showed a significant positive correlation with both bacterial and fungal community composition, yet the extent of influence on these two communities varied (Table 2). These findings align with our initial hypothesis and are consistent with previous research on other plant species (Hunter et al., 2010; Wei et al., 2022; Gao et al., 2023). The disparities in bacterial and fungal community responses may be attributed to variations in stoichiometry, enzyme function, and carbon utilization mechanisms among bacteria and fungi. Results from VPA and random forest modeling further underscored that fungal community composition was more impacted by plant trait compared with bacterial community composition (Figs. 7 and 8). Taken together, these results indicated that fungi were more responsive to changes in plant characteristics than bacteria.

5 Conclusions

Diversity of plant epiphytic microbial communities in natural ecosystems has attracted increasing interest from researchers. This study is the first to reveal the presence of multiple types of epiphytic microbial communities, including bacteria and fungi, on the leaves of plants in arid environments. Phyllosphere epiphytic bacterial community of different plant life forms was mainly consisted of Proteobacteria, Cyanobacteria, Actinobacteriota, and Firmicutes, while fungal community was primarily composed of Ascomycota and Basidiomycota. Co-occurrence network analysis revealed that complexity of bacterial community network varied among different plant life forms, with Proteobacteria and Cyanobacteria showing the highest positive correlations and Firmicutes showing the highest negative correlation. Ascomycota and Basidiomycota had the highest positive correlations among fungal communities, while Chytridiomycota showed the highest negative correlation. Our study revealed that numerous microorganisms are likely involved in material cycling and energy flow within ecosystems, enhancing our comprehension of microbial community diversity in arid deserts and shedding light on the potential ecological roles of epiphytic microbial communities. Furthermore, we observed that phyllosphere fungal community of desert plants was more responsive to variations in leaf functional traits than bacterial community. These results underscore the significance of microbial characteristics in governing ecological processes and adaptations to changes in plant attributes. Consequently, these findings could enhance our capacity to forecast the reaction of plant microbiomes and their ecosystem functions to environmental shifts.

Conflict of interest

ZHANG Yuanming is an editorial board member of Journal of Arid Land and was not involved in the editorial review or the decision to publish this article. All authors declare that there are no competing interests.

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Author contribution

Conceptualization: ZHANG Jun, ZHANG Yuanming; Methodology: ZHANG Jun, ZHANG Qi; Investigation: ZHANG Jun, ZHANG Qi; Writing-original draft preparation: ZHANG Jun; Writing - review and editing: ZHANG Jun, ZHANG Yuanming; Funding acquisition: ZHANG Jun, ZHANG Yuanming; Supervision: ZHANG Jun, ZHANG Yuanming. All authors approved the manuscript.

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Appendix

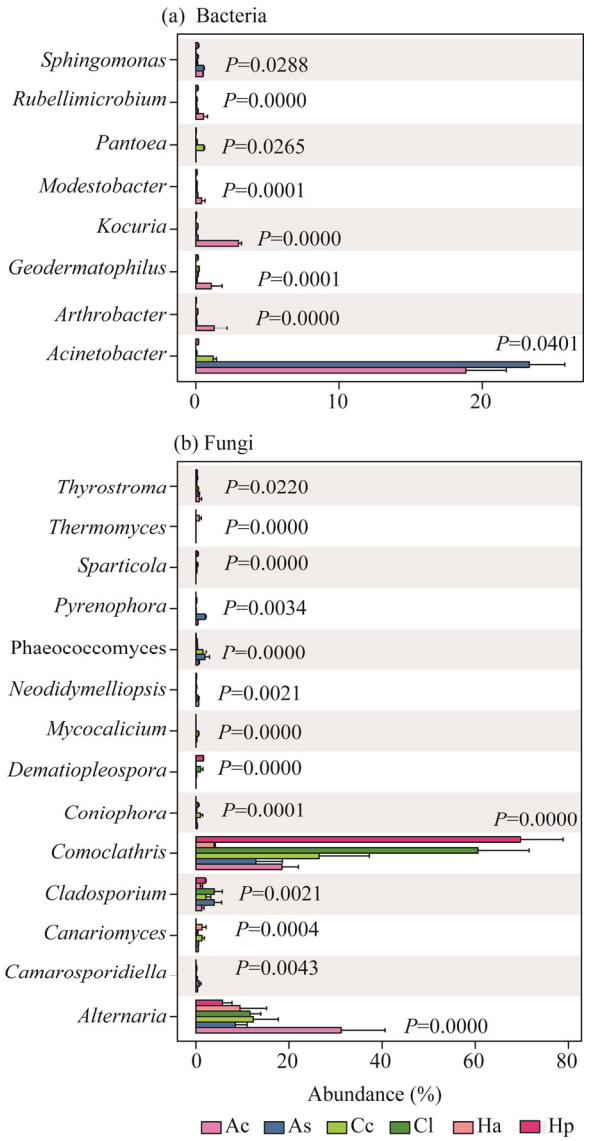

Fig. S1 Abundance of epiphytic bacterial communities (a) and fungal communities (b) of different plant species. Bars are standard errors. Ac, *Astragalus cognatus* Schrenk; As, *Astragalus steinbergianus* Sumn; Cc, *Calligonum caput-medusae* Schrenk; Cl, *Calligonum leucocladum* (Schrenk) Bunge; Ha, *Haloxylon ammodendron* (C. A. Mey.) Bunge; Hp, *Haloxylon periscum* Bunge ex Boiss & Buhse. The abbreviations are the same as in the following figures and tables.

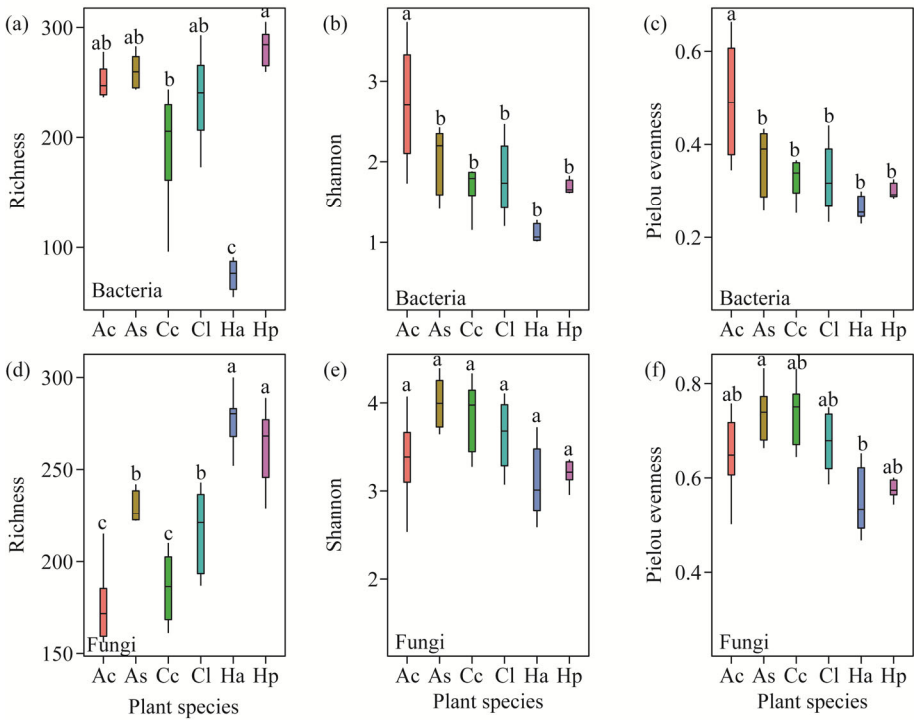

Fig. S2 Alpha diversity of epiphytic bacterial communities (a–c) and fungal communities (d–f) of different plant species. Different lowercase letters indicate significant differences among different plant species at $P<0.050$ level. Boxes indicate the IQR (interquartile range, 75th to 25th of the data). The median value is shown as a line within the box. Lines extend to the most extreme value within 1.5×IQR.

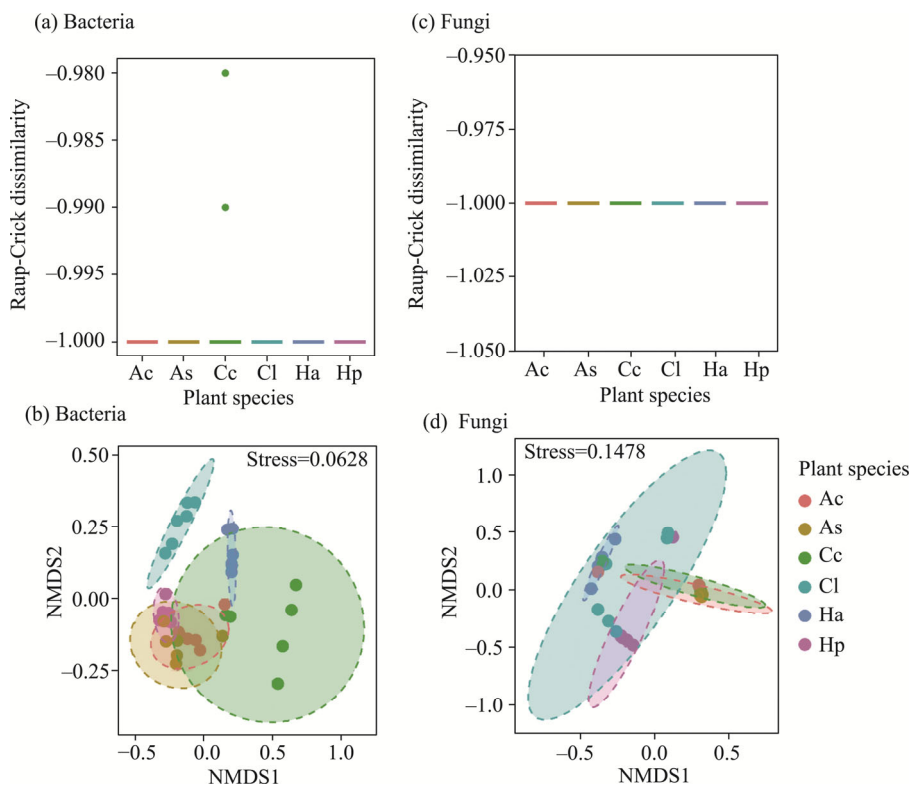

Fig. S3 Raup-Crick dissimilarity index and non-metric multi-dimensional dissimilarity (NMDS) index of phyllosphere epiphytic bacterial communities (a and b) and fungal communities (c and d) of different plant species. Outlier is shown as circle in Figure S3a and c.

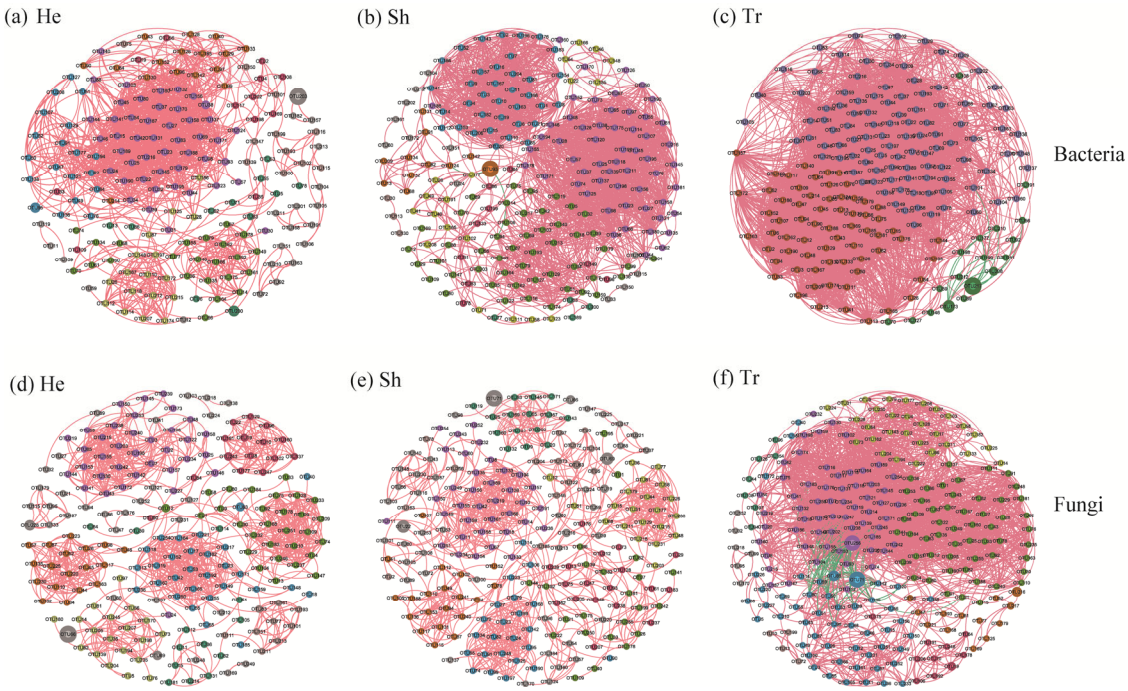

Fig. S4 Co-occurrence networks of phyllosphere epiphytic bacteria (a–c) and fungi (d–f) of different plant life forms. He, herb; Sh, shrub; Tr, tree.

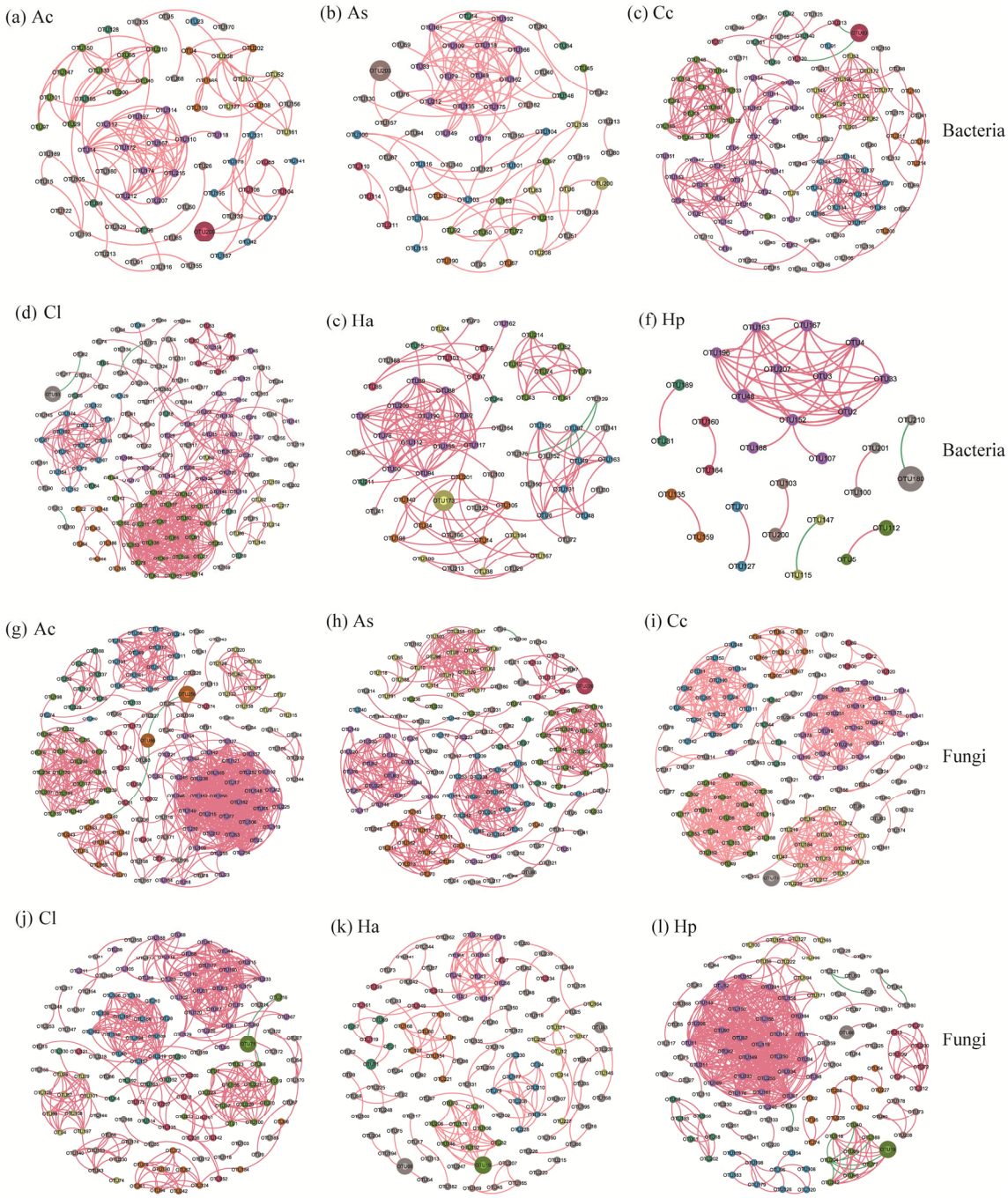

Fig. S5 Co-occurrence networks of phyllosphere epiphytic bacteria (a–f) and fungi (g–l) of different plant species

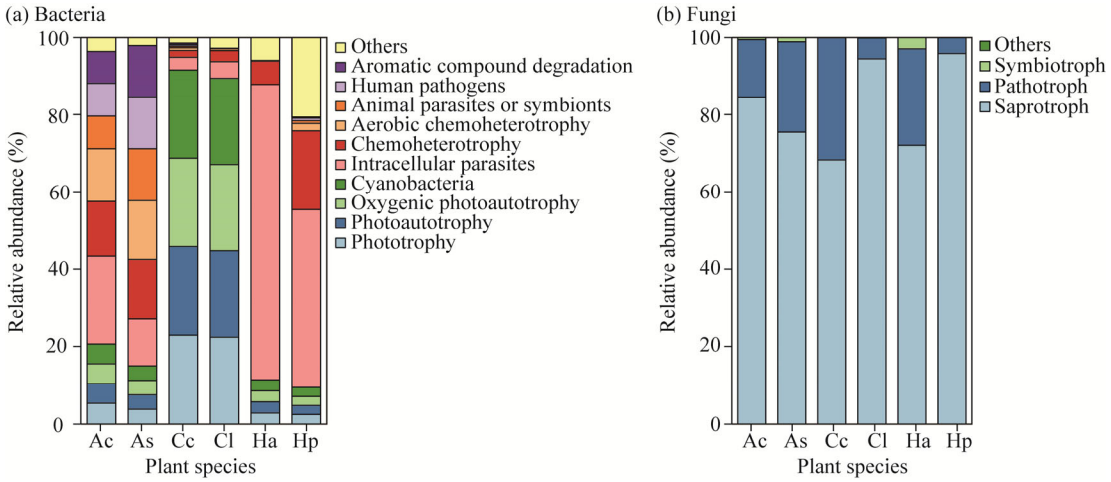

Fig. S6 Function prediction of phyllosphere epiphytic bacterial communities (a) and fungal communities (b) of different plant species

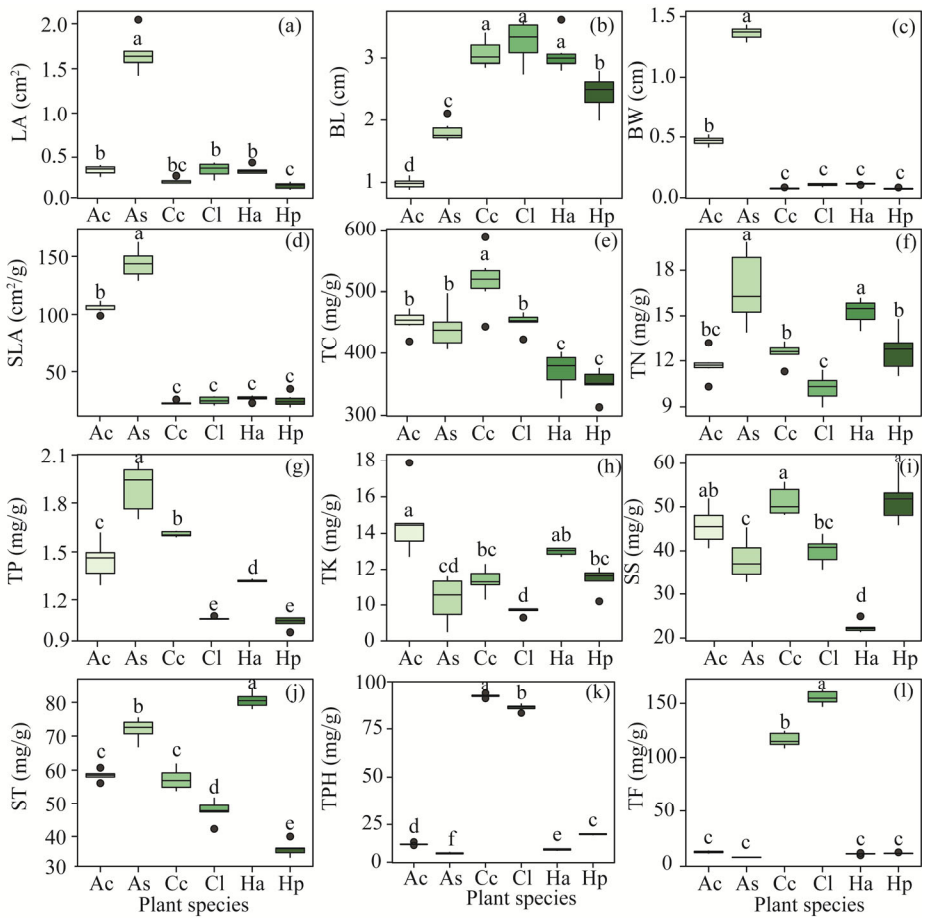

Fig. S7 Functional traits of different plant leaves. Different lowercase letters indicate significant differences among different plant species at $P<0.050$ level. (a), LA (leaf area); (b), BL (blade length); (c), BW (blade width); (d), SLA (specific leaf area); (e), TC (total carbon); (f), TN (total nitrogen); (g), TP (total phosphorus); (h), TK (total potassium); (i), SS (soluble sugar); (j), ST (starch); (k), TPH (total phenol); (l), TF (total flavone). Boxes indicate the IQR (interquartile range, 75th to 25th of the data). The median value is shown as a line within the box. Lines extend to the most extreme value within 1.5×IQR. Outlier is shown as circle.

Table S1 Characteristics of co-occurrence networks of phyllosphere epiphytic microorganisms of different plant life forms

Bacterial network topological characteristics	He	Sh	Tr	Fungal network topological characteristics	He	Sh	Tr
Total node	177	199	187	Total node	198	215	235
Total edge	1327	2087	6573	Total edge	945	898	3261
Positive edge percentage (%)	100.00	99.90	99.82	Positive edge percentage (%)	100.00	100.00	97.76
Average degree	14.994	20.975	70.299	Average degree	9.545	8.353	27.753
Average weighted degree	12.919	18.333	57.289	Average weighted degree	8.627	7.431	23.608
Average clustering coefficient	0.705	0.743	0.730	Average clustering coefficient	0.732	0.691	0.701
Average path length	4.462	3.985	1.809	Average path length	6.360	8.095	3.171
Modularity	0.464	0.572	0.206	Modularity	0.820	0.816	0.450
Number of weakly connected component	7	8	1	Number of weakly connected components	9	3	4
Network diameter	13	11	5	Network diameter	16	22	12
Graph density	8.5	10.6	37.8	Graph density	4.8	3.9	11.9

Note: He, herb; Sh, shrub; Tr, tree.

Table S2 Characteristics of co-occurrence networks of phyllosphere epiphytic microorganisms of different plant species

Network topological characteristics	Ac	As	Cc	Cl	Ha	Hp
Bacteria						
Total node	68	63	103	136	63	30
Total edge	136	121	284	479	154	55
Positive edges percentage (%)	100.00	100.00	99.30	99.37	98.70	96.36
Average degree	4.000	3.841	5.515	7.044	4.889	3.667
Average weighted degree	3.974	3.816	5.471	6.949	4.868	3.655
Average clustering coefficient	0.830	0.779	0.843	0.687	0.971	0.940
Average path length	1.678	1.587	1.741	2.649	1.116	1.293
Modularity	0.768	0.624	0.791	0.590	0.764	0.297
Number of weakly connected component	14	16	20	22	14	10
Network diameter	6	4	5	7	3	3
Graph density	6.0	6.2	5.4	5.2	7.9	12.6
Fungi						
Total node	136	134	121	131	115	118
Total edge	780	478	575	456	176	517
Positive edges percentage (%)	99.74	99.79	100.00	99.56	100.00	97.49
Average degree	11.471	7.134	9.504	6.692	3.061	8.763
Average weighted degree	11.307	7.054	9.402	6.886	3.042	8.683
Average clustering coefficient	0.811	0.763	0.928	0.782	0.766	0.833
Average path length	2.098	1.790	1.217	1.895	1.296	1.832
Modularity	0.620	0.828	0.785	0.768	0.885	0.418
Number of weakly connected component	10	18	21	18	33	23
Network diameter	7	6	4	6	4	6
Graph density	8.5	5.4	7.9	5.4	2.7	7.5